

An Agency of Industry Canada Office de la Propri,t, Intellectuelle du Canada

Un organisme d'Industrie Canada (11) CA 2 331 641

(13) A1

(40) 11.11.1999 (43) 11.11.1999

(12)

(21) 2 331 641

(22) 05.05.1999

(51) Int. Cl. 6:

C07K 16/00, C07K 16/28, A61K 39/395, G01N 33/53,

C12N 15/63

(85) 03.11.2000

(86) PCT/DE99/01350

(30) 198 19 846.9 DE 05.05.1998

71)
DEUTSCHES KREBSFORSCHUNGSZENTRUM
STIFTUNG DES ***FFENTLICHEN RECHTS,
Im Neuenheimer Feld 280, HEIDELBERG, XX (DE).

(87) WO99/57150 (72)

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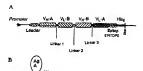
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(54) CONSTRUCTIONS D'ANTICORPS MULTIVALENTES

(54) MULTIVALENT ANTIBODY CONSTRUCTS

(57)

The invention relates to a multivalent Fv antibody, construct comprising at least four variable domains which are connected to one another via peptide linkers in J. 2 and 3. The invention also relates to expression plasmids which code for such an Fv antibody construct in addition, the invention relates to a method for producing the Fv antibody constructs and to the use thereof.







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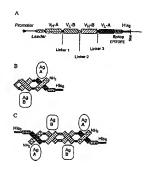
(2D/AD 2.331.641

(86) 1999/05/05 (87) 1999/11/11

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- (71) DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DES ÖFFENTLICHEN RECHTS, DE
- (51) Int.CL6 C07K 16/00, C12N 15/63, G01N 33/53, A61K 39/395. C07K 16/28
- (30) 1998/05/05 (198 19 846.9) DE
- (54) CONSTRUCTIONS D'ANTICORPS MULTIVALENTES
- (54) MULTIVALENT ANTIBODY CONSTRUCTS



(57) La présente invention concerne une construction d'anticorps F, multivalente, comportant au moins quatre domaines variables qui sont reliés l'un à l'autre par l'intermédiaire des segments peptidiques 1, 2 et 3. L'invention concerne en outre des plasmides d'expression qui codent pour une telle construction d'anticorps F_v, ainsi qu'un procédé de réalisation des constructions d'anticorps F, et leur utilisation

(57) The invention relates to a multivalent F, antibody construct comprising at least four variable domains which are connected to one another via peptide linkers 1, 2 and 3. The invention also relates to expression plasmids which code for such an F, antibody construct. In addition, the invention relates to a method for producing the F, antibody constructs and to the use thereof.

WELTORGANISATION FÜR GEISTIGES EIGENTUM Internationales Büro PCT

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 6: (11) Internationale Veröffentlichungsnummer: WO 99/57150 C07K 16/00 A2 (43) Internationales Veröffentlichungsdatum: 11. November 1999 (11.11.99) (21) Internationales Aktenzeichen: PCT/DE99/01350 (81) Bestimmungsstaaten: AL, AM, AT, AU, AZ, BA, BB, BG. BR, BY, CA, CH, CN, CU, CZ, DK, EE, ES, FI, GB, GD. (22) Internationales Anmeldedatum: GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, 5. Mai 1999 (05.05.99) KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, (30) Prioritätsdaten: 198 19 846.9 5. Mai 1998 (05.05.98) DF ARIPO Patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, (71) Anmelder (für alle Bestimmungsstaaten ausser US): ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DES ÖFFENTLICHEN RECHTS [DE/DE]; NE, SN, TD, TG). Im Neuenheimer Feld 280, D-69120 Heidelberg (DE).

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Veröffentlicht

Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.

(54) Title: MULTIVALENT ANTIBODY CONSTRUCTS

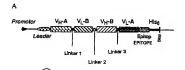
(54) Bezeichnung: MULTIVALENTE ANTIKÖRPER-KONSTRUKTE

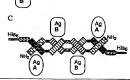
(57) Abstract

The invention relates to a multivalent Fv antibody construct comprising at least four variable domains which are connected to one another via peptide linkers 1, 2 and 3. The invention also relates to expression plasmids which code for such an F, antibody construct. In addition, the invention relates to a method for producing the F, antibody constructs and to the use thereof.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft ein multivalentes Fy-Antikörper-Konstrukt mit mindestens vier variablen Domänen, die über die Peptidlinker 1, 2 und 3 miteinander verbunden sind. Ferner betrifft die Erfindung Expressionsplasmide, die für ein solches Fy-Antikorper-Konstrukt codieren, und ein Verfahren zur Herstellung der Fy-Antikörper-Konstrukte sowie deren Verwendung.





CA 02331641 2000-11-03

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Multivalent Antibody Constructs

The present invention relates to multivalent F_{ν} antibody constructs, expression plasmids which code for them, and a method for producing the F_{ν} antibody constructs as well as the use thereof.

Natural antibodies are dimers and are therefore referred to as bivalent. They have four variable domains, namely two $V_{\rm K}$ domains and two $V_{\rm L}$ domains. The variable domains serve as binding sites for an antigen, a binding site being formed from a $V_{\rm H}$ domain and a $V_{\rm L}$ domain. Natural antibodies recognize one antigen each, so that they are also referred to as monospecific. Furthermore, they also have constant domains which add to the stability of the natural antibodies. On the other hand, they are also co-responsible for undesired immune responses which result when natural antibodies of various animal species are administered mutually.

In order to avoid such immune responses, antibodies are constructed which lack the constant domains. In particular, these are antibodies which only comprise the variable domains. Such antibodies are designated F_{ν} antibody constructs. They are often available in the form of single-chain monomers paired with one another.

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However, it showed that F_{ν} antibody constructs only have little stability. Therefore, their usability for therapeutic purposes is strongly limited.

Thus, it is the object of the present invention to provide an antibody by means of which undesired immune responses can be avoided. Furthermore, it shall have a stability which makes it usable for therapeutic uses.

According to the invention this is achieved by the subject matters defined in the claims.

Therefore, the subject matter of the present invention relates to a multivalent F_{ν} antibody construct which has great stability. Such a construct is suitable for diagnostic and therapeutic purposes.

The present invention is based on the applicant's insights that the stability of an F_v antibody construct can be increased if it is present in the form of a single-chain dimer where the four variable domains are linked with one another via three peptide linkers. The applicant also recognized that the F_v antibody construct folds with itself when the middle peptide linker has a length of about 10 to 30 amino acids. The applicant also recognized that the F_v antibody constructs when the middle peptide linker has a length of about up to 10 amino acids so as to obtain a multimeric, i.e. multivalent, F_v antibody construct. The applicant also realized that the F_v antibody construct can be multi-specific.

According to the invention the applicant's insights are utilized to provide a multi-valent F_{ν} antibody construct

which comprises at least four variable domains which are linked with one another via peptide linkers 1. 2 and 3.

The expression " F_{ν} antibody construct" refers to an antibody which has variable domains but no constant domains.

The expression "multivalent F_{ν} antibody construct" refers to an F_v antibody which has several, but at least four, variable domains. This is achieved when the single-chain F_{ν} antibody construct folds with itself so as to give four variable domains, or folds with other single-chain F., antibody constructs. In the latter case, an F_{ν} antibody construct is given which has 8, 12, 16, etc., variable domains. It is favorable for the F_{ν} antibody construct to have four or eight variable domains, i.e. it is bivalent or tetravalent (cf. Fig. 1). Furthermore, the variable domains may be equal or differ from one another, so that the antibody construct recognizes one or several antigens. The antibody construct preferably recognizes one or two antigens, i.e. it is monospecific and bispecific, respectively. Examples of such antigens are proteins CD19 and CD3.

The expression "peptide linkers 1, 3" refers to a peptide linker adapted to link variable domains of an F_{ν} antibody construct with one another. The peptide linker may contain any amino acids, the amino acids glycine (G), serine (S) and proline (P) being preferred. The peptide linkers 1 and 3 may be equal or differ from each other. Furthermore, the peptide linker may have a length of about 0 to 10 amino acids. In the former case, the peptide linker is only a peptide bond from the COOH residue of one of the variable domains and the NHz residue of another of the variable domains. The peptide linker preferably comprises the amino acid sequence GG.

The expression "peptide linker 2" refers to a peptide linker adapted to link variable domains of an F_{ν} antibody construct with one another. The peptide linker may contain any amino acids, the amino acids glycine (G), serine (S) and proline (P) being preferred. The peptide linker may also have a length of about 3 to 10 amino acids, in particular 5 amino acids, and most particularly the amino acid sequence GGPGS, which serves for achieving that the single-chain F_{ν} antibody construct folds with other single-chain F_{ν} antibody constructs. The peptide linker can also have a length of about 11 to 20 amino acids, in particular 15 to 20 amino acids, and most particularly the amino acid sequence $(G_{\nu}S)_{+\nu}$, which serves for achieving that the single-chain F_{ν} antibody construct folds with itself.

An F_{ν} antibody construct according to the invention can be produced by common methods. A method is favorable in which DNAs coding for the peptide linkers 1, 2 and 3 are ligated with DNAs coding for the four variable domains of an F_{ν} antibody construct such that the peptide linkers link the variable domains with one another and the resulting DNA molecule is expressed in an expression plasmid. Reference is made to Examples 1 to 6. As to the expressions " F_{ν} antibody construct" and "peptide linker" reference is made to the above explanations and, by way of supplement, to Maniatis, T. et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory 1982.

DNAs which code for an F_v antibody construct according to the invention also represent a subject matter of the present invention. Furthermore, expression plasmids which contain such DNAs also represent a subject matter of the present invention. Preferred expression plasmids are pDISC3x19-LL,

pDISC3x19-SL, pPIC-DISC-LL, pPIC-DISC-SL, pDISC5-LL and pDISC6-SL. The first four were deposited with the DSMZ (Deutsche Sammlung für Mikroorganismen und Zellen) [Germantype collection for micro-organisms and cells] on April 30, 1998 under DSM 12150, DSM 12149, DSM 12152 and DSM 12151, respectively.

Another subject matter of the present invention relates to a kit, comprising:

- (a) an F_{ν} antibody construct according to the invention, and/or
- (b) an expression plasmid according to the invention, and
- (c) conventional auxiliary agents, such as buffers, solvents and controls.

One or several representatives of the individual components may be present.

The present invention provides a multivalent F_{ν} antibody construct where the variable domains are linked with one another via peptide linkers. Such an antibody construct distinguishes itself in that it contains no parts which can lead to undesired immune reactions. Furthermore, it has great stability. It also enables to bind several antigens simultaneously. Therefore, the F_{ν} antibody construct according to the invention is perfectly adapted to be used not only for diagnostic but also for therapeutic purposes. Such purposes can be seen as regards any disease, in particular a viral, bacterial or tumoral disease.

Brief description of the drawings:

- Fig. 1 shows the genetic organization of an F_{ν} antibody construct (A) according to the invention and schemes for forming a bivalent (B) or tetravalent F_{ν} antibody construct (C). Ag: antigen; His,: six C-terminal histidine residues; stop: stop codon (TAA); V_{ii} and $V_{i:}$ variable region of the heavy and light chains.
- Fig. 2 shows the scheme for the construction of the plasmids pDISC3x19-LL and pDISC3x19-SL. c-myc: sequence coding for an epitope which is recognized by the antibody 9El, $\operatorname{His}_{\epsilon}$: sequence which codes for six C-terminal histidine residues; PelB: signal peptide sequence of the bacterial pectate lyase (PelB leader); rbs: ribosome binding site; Stop: stop codon (TAA); V_{H} and V_{L} : variable region of the heavy and light chains.
- Fig. 3 shows a diagram of the expression plasmid pDISC3x19-LL. 6xHis: sequence which codes for six C-terminal histidine residues; bla: gene which codes for B-lactamase responsible for ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; ColE1: origin of the DNA replication; f1-IG: intergenic region of the bacteriophage f1; Lac P/O: wt lacoperon promoter/operator; linker 1: sequence which codes for a GlyGly dipeptide linking the V_B and V_L domains; linker 2: sequence coding for a (Gly4Ser)4 polypeptide which links the hybrid scFv fragments; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site; V_B and V_L : variable region of the heavy and light chains.
- Fig. 4 shows a diagram of the expression plasmid pDISC3x19-SL. 6xHis: sequence which codes for six C-terminal histidine

residues; bla: gene which codes for 3-lactamase which is responsible for the ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope recognized by the 9E10 antibody; ColE1: origin of DNA replication; f1-IG: intergenic region of the bacteriophage f1; Lac P/O: wt lacoperon promoter/operator: linker 1: sequence which codes for a GlyGly dipeptide which links the $V_{\rm H}$ and $V_{\rm L}$ domains; linker 3: sequence which codes for a GlyGlyProGlySer oligopeptide which links the hybrid scFv fragments; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site; $V_{\rm H}$ and $V_{\rm L}$: variable region of the heavy and light chains.

Fig. 5 shows the nucleotide sequence and the amino acid sequence derived therefrom of the bivalent F_{ν} antibody construct encoded by the expression plasmid pDIS3x19-LL. c-myc epitope: sequence coding for an epitope which is recognized by the antibody 9E10; CDR: region determining the complementarity; framework: framework region; His6 tail: sequence which codes for six C-terminal histidine residues; PelB leader: signal peptide sequence of the bacterial pectate lyase; RBS: ribosome binding site; V_{H} and V_{L} : variable region of the heavy and light chains.

Fig. 6 shows the nucleotide sequence and the derived amino acid sequence of the tetravalent F_{ν} antibody construct encoded by the expression plasmid pDISC3x19-SL. c-myc epitope: sequence coding for an epitope which is recognized by the 9E10 antibody; CDR: region determining complementarity; framework: framework region; His6 tail: sequence coding for the six C-terminal histidine residues; PelB leader: signal peptide sequence of the bacterial pectate lyase; RBS: ribosome binding site; V_{H} and V_{L} : variable region of the heavy and light chains.

Fig. 7 shows the nucleotide sequence and the derived amino acid sequence of a connection between a gene which codes for an α -factor leader sequence and a gene coding for the tetravalent F_{ν} antibody construct in the Pichia expression plasmid pPIC-DISC-SL. Alpha-factor signal: leader peptide sequence of the Saccharomyces cerevisiae- α factor secretion signal; $V_{\rm H}$: variable region of the heavy chain. Rhombs indicate the signal cleaving sites.

Fig. 8 shows the nucleotide sequence and the derived amino acid sequence of a connection between a gene coding for an α -factor leader sequence and a gene which codes for the bivalent F_{ν} antibody construct in the *Pichia* expression plasmid pPIC-DISC-LL. Alpha-factor signal: leader peptide sequence of the *Saccharomyces cerevisiae-\alpha* factor secretion signal; $V_{\rm H}$: variable region of the heavy chain. Rhombs show the signal cleaving sites.

Fig. 9 shows a diagram of the expression plasmid pDISC5-LL. 6xHis: sequence coding for six C-terminal histidine residues; bla: gene which codes for β-lactamase responsible for ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; hok-sok: plasmid-stabilizing DNA locus; LacI: gene which codes for the Lac repressor; Lac P/O: wt lac-operon-promoter/operator; LacZ': gene which codes for the α-peptide of β-galactosidase; linker 1: sequence which codes for a GlyGly dipeptide connecting the V_H and V_L domains; linker 2: sequence which codes for a (Gly4Ser)4 polypeptide linking the hybrid scFv fragments; M13 IG: intergenic region of the M13 bacteriophage; pBR322ori: origin of DNA replication; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site which originates

from the E. coli lacZ gene (lacZ), from the bacteriophage T7 gene 10 (T7g10) or from the E. coli skp gene (skp); skp: gene which codes for the bacterial periplasmic factor Skp/OmpH; tHP: strong transcription terminator; tIPP: transcription terminator; $V_{\rm H}$ and $V_{\rm L}$: variable region of the heavy and light chains.

Fig. 10 shows a diagram of the expression plasmid pDISC6-SL. 6xHis: sequence which codes for six C-terminal histidine residues; bla: gene which codes for ß-lactamase responsible for ampicillin resistance; bp: base pairs: c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; hok-sok: plasmid-stabilized DNA locus; LacI: gene which codes for the Lac repressor; Lac P/O: wt lac-operon promoter/operator; LacZ': gene which codes for the α -peptide of ß-galactosidase; linker 1: sequence which codes for a GlyGly dipeptide which links the $V_{\rm H}$ and $V_{\rm L}$ domains; linker 3: sequence which codes for a GlyGlyProGlySer oligopeptide linking the hybrid scFv fragments: M13 IG: intergenic region of the M13 bacteriophage; pBR322ori: origin of DNA replication; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site originating from the E. coli lacZ gene (lacZ), from the bacteriophage T7 gene 10 (T7g10) or from the E. coli skp gene (skp); skp: gene which codes for the bacterial periplasmic factor Skp/OmpH; tHP: strong transcription terminator; tIPP: transcription terminator; V_H and V_L : variable region of the heavy and light chains.

The invention is explained by the below examples.

Example 1: Construction of the plasmids pDISC3x19-LL and pDISC3x19-SL for the expression of bivalent, bispecific and/or tetravalent, bispecific F_v antibody constructs in bacteria

The plasmids pHOG- α CD19 and pHOG-dmOKT3 which code for the scFv fragments derived from the hybridoma HD37 which is specific to human CD19 (Kipriyanov et al., 1996, J.-Immunol. Meth. 196, 51-62) and from the hybridoma OKT3 which is specific to human CD3 (Kipriyanov et al., 1997, Protein Eng. 10, 445-453), respectively, were used for the construction of expression plasmids for a single-chain F_{ν} antibody construct. A PCR fragment 1 of the V_{H} domain of anti-CD19, followed by a segment which codes for a GlyGly linker, was produced using the primers DP1, 5'-TCACACAGAATTC-TTAGATCTATTAAAGAGGGAGAAATTAACC, and DP2, 5'-AGCACACGATATCACCGCCAAGCTTGGGTGTTGTTTTGGC (cf. Fig. 2). The PCR fragment 1 was cleaved by EcoRI and EcoRV and ligated with the EcoRI/EcoRV-linearized plasmid pHOG-dmOKT3 so as to produce the vector pHOG19-3. The PCR fragment 2 of the $V_{\rm L}$ domain of anti-CD19, followed by a segment which codes for a c-myc epitope and a hexahistidinyl tail, was produced using the primers DP3, 5'-AGCACACAAGCTTGGCGGTGATATCTTGCTCACCCAAAC-TCCA, and DP4, 5'-AGCACACTCTAGAGACACAGATCTTTAGTGATGGTGAT-GGTGATGTGAGTTTAGG. The PCR fragment 2 was cleaved by HindIII and XbaI and ligated with the HIndIII/XbaI-linearized plasmid pHOG-dmOKT3 so as to obtain the vector pHOG3-19 (cf. Fig. 2). The gene coding for the hybrid scFv-3-19 in the plasmid pHOG3-19 was amplified by means of PCR with the primers Bi3sk, 5'-CAGCCGGCCATGGCGCAGGTGCAACTGCAGCAG and either Li-1, 5'-TATATACTGCAGCTGCACCTGGCTACCACCACCACCGGAGCCG-for the production of a long flexible (Gly4Ser)4 inter-scFV linker (PCR fragment 3, cf. Fig. 2) or Li-2, 5'-TATATA- CTGCAGCTGCACCTGGGCCACCAGCGGCCGACCAGCAGCATCAGCCCG, for the production of a short rigid GGPGS linker (PCR fragment 4, cf. Fig. 2). The expression plasmids pDISC3x19-LL and pDISC3x19-SL were constructed by ligating the NcoI/PvuII restriction fragment from pHOG19-3, comprising the vector framework and the NcoI/PvuII-cleaved PCR fragments 3 and 4, respectively (cf. Figs. 3, 4). The complete nucleotide and protein sequences of the bivalent and tetravalent $F_{\rm v}$ antibody constructs are indicated in Figs 5 and 6, respectively.

Example 2: Construction of the plasmids pPIC-DISC-LL and pPIC-DISC-SL for the expression of bivalent, bispecific and/or tetravalent, bispecific F_{ν} antibody constructs in yeast

(A) Construction of pPIC-DISC-SL

The vector pPIC2αA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins in the yeast *Pichia pastoris* was used as a starting material. It contains a gene which codes for the *Saccharomyces cerevisiae* α-factor secretion signal, followed by a polylinker. The secretion of this vector is based on the dominant selectable marker, ZeocinTM which is bifunctional in both *Pichia* and *E. coli*. The gene which codes for the tetravalent F_v antibody the template pDISC3x19-SL using the primers 5-PIC, 5'-CCCTGAATTCCAGGTGCAACTGCAGCTGCAGCTGAACTGGC, and pSEXBn 5'-GGTCGACCTTAACCGACAACACAGATAAAACG. The resulting PCR product was cleaved by EcoRI and XbaI and ligated in EcoRI/XbaI-linearized pPICZαA. The expression plasmid pPICDISC-SL was obtained. The nucleotide and protein sequences

of the tetravalent F_{ν} antibody construct are shown in Fig. 7.

(B) Construction of pPIC-DISC-LL

The construction of pPIC-DISC-LL was carried out on the basis of pPIC2 α A (Invitrogen BV, Leek, Netherlands) and pDISC3x19-LL (cf. Fig. 3). The plasmid-DNA pPIC2 α A was cleaved by EcoRI. The overhanging 5'-ends were filled using a Klenow fragment of the E. coli DNA polymerase I. The resulting DNA was cleaved by XbaI, and the large fragment comprising the pPIC vector was isolated. Analogous thereto the DNA of pDISC3x19-LL was cleaved by NcoI and treated with a Klenow fragment. Following the cleavage using XbaI a small fragment, comprising a gene coding for the bivalent F_v antibody, was isolated. Its ligation with a pPIC-derived vector-DNA resulted in the plasmid pPIC-DISC-LL. The nucleotide and protein sequences of the bivalent F_v antibody construct are shown in Fig. 8.

Example 3: Expression of the tetravalent and/or bivalent F_v antibody construct in bacteria

E. coli XL1-blue cells (Strategene, La Jolla, CA) which had been transformed with the expression plasmids pDISC3x19-LL and pDISC3x19-SL, respectively, were cultured overnight in 2xYT medium with 50 μ g/ml ampicillin and 100 mM glucose (2xYT_{Ga}) at 37°C. 1:50 dilutions of the overnight cultures in 2xYT_{Ga} were cultured as flask cultures at 37°C while shaking with 200 rpm. When the cultures had reached an OD₆₀₀ value of 0.8, the bacteria were pelleted by 10-minute centrifugation with 1500 g at 20°C and resuspended in the same volume of a fresh 2xYT medium containing 50 μ g/ml ampicillin and 0.4 M saccharose. IPTG was added up to a

final concentration of 0.1 mM, and the growth was continued at room temperature (20-22°C) for 18 - 20 h. The cells were harvested by 10-minute centrifugation with 5000 g at 4°C. The culture supernatant was held back and stored on ice. In order to isolate the soluble periplasmic proteins, the pelleted bacteria were resuspended in 5 % of the initial volume of ice-cold 50 mM Tris-HCl, 20 % saccharose, 1 mM EDTA, pH 8.0. Following 1 hour of incubation on lice with occasional stirring the spheroplasts were centrifuged with 30,000 g at 4°C for 30 minutes, the soluble periplasmic extract being obtained as supernatant and the spheroplasts with the insoluble periplasmic material being obtained as pellet. The culture supernatant and the soluble periplasmic extract were combined and clarified by further centrifugation (30,000 g, 4°C, 40 min.). The recombinant product was concentrated by ammonium sulfate precipitation (final concentration 70 % saturation). The protein precipitate was obtained by centrifugation (10,000 g, 4°C, 40 min.) and dissolved in 10 % of the initial volume of 50 mM Tris-HCl, 1 M NaCl, pH 7.0. An immobilized metal affinity chromatography (IMAC) was carried out at 4°C using a 5 ml column of chelating sepharose (Pharmacia) which was charged with Cu2+ and had been equilibrated with 50 mM Tris-HCl, 1 M NaCl, pH 7.0 (starting buffer). The sample was loaded by passing it over the column. It was then washed with twenty column volumes of starting buffer, followed by starting buffer with 50 mM imidazole until the absorption at 280 nm of the effluent was at a minimum (about thirty column volumes). The absorbed material was eluted with 50 mM Tris-HCl, 1 M NaCl, 250 mM imidazole, pH 7.0.

The protein concentrations were determined with the Bradford dye binding test (1976, Anal. Biochem. 72, 248-254) using the Bio-Rad (Munich, Germany) protein assay kit. The

concentrations of the purified tetravalent and bivalent F_{ν} antibody constructs were determined from the A_{290} values using the extinction coefficients $\epsilon^{1mg/ml}=1.96$ and 1.93, respectively.

Example 4: Expression of the tetravalent and/or bivalent antibody construct in the yeast Pichia pastoris

Competent *P. pastoris* GS155 cells (Invitrogen) were electroporated in the presence of 10 µg plasmid-DNA of pPIC-DISC-LL and pPIC-DISC-SL, respectively, which had been linearized with SacI. The transformants were selected for 3 days at 30°C on YPD plates containing 100 µg/ml ZeocinTM. The clones which secreted the bivalent and/or tetravalent F_V antibody constructs were selected by plate screening using an anti-c-myc-mAk 9E10 (IC Chemikalien, Ismaning, Germany).

For the expression of the bivalent F_{ν} antibody constructs and tetravalent F_{ν} antibody constructs, respectively, the clones were cultured in YPD medium in shaking flasks for 2 days at 30°C with stirring. The cells were centrifuged resuspended in the same volume of the medium containing methanol and incubated for another 3 days at 30°C with stirring. The supernatants were obtained after the centrifugation. The recombinant product was isolated by ammonium sulfate precipitation, followed by IMAC as described above.

Example 5: Characterization of the tetravalent F_{ν} antibody construct and bivalent F_{ν} antibody construct, respectively,

(A) Size exclusion chromatography

An analytical gel filtration of the F_{ν} antibody constructs was carried out in PBS using a superdex 200-HR10/30 column (Pharmacia). The sample volume and the flow rate were 200 μ l/min and 0.5 ml/min, respectively. The column was calibrated with high-molecular and low-molecular gel filtration calibration kits (Pharmacia).

(B) Flow cytometry

The human CD3+/CD19-acute T-cell leukemia line Jurkat and the CD19*/CD3 B-cell line JOK-1 were used for flow cytometrie. 5 x 10^5 cells in 50 μl RPMI 1640 medium (GIBCO BRL, Eggestein, Germany) which was supplemented with 10 % FCS and 0.1 % sodium azide (referred to as complete medium) were incubated with 100 μl of the F_{ν} antibody preparations for 45 minutes on ice. After washing using the complete medium the cells were incubated with 100 µl 10 µg/ml anti-cmyc-Mak 9E10 (IC Chemikalien) in the same buffer for 45 min on ice. After a second wash cycle, the cells were incubated with 100 ul of the FITC-labeled goat-anti-mouse-IgG (GIBCO BRL) under the same conditions as before. The cells were then washed again and resuspended in 100 µl 1 µg/ml propidium iodide solution (Sigma, Deisenhofen, Germany) in complete medium with the exclusion of dead cells. The relative fluorescence of the stained cells was measured using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

(C) Cytotoxicity test

The CD19-expressing Burkitt lymphoma cell line Raji and Namalwa were used as target cells. The cells were incubated in RPMI 1640 (GIBCO BRL) which was supplemented with 10^{-8}

heat-inactivated FCS (GIBCO BRL), 2 mM glutamine and 1 mM pyruvate, at 37°C in a dampened atmosphere with 7.5 % CO2. The cytotoxic T-cell tests were carried out in RPMI-1640 medium supplemented with 10 % FCS, 10 mM HEPES, 2 mM glutamine, 1 mM pyruvate and 0.05 mM 2-ME. The cytotoxic activity was evaluated using a standard[51Cr] release test; 2 x 10 6 target cells were labeled with 200 $\mu\text{Ci Na}\,[^{51}\text{Cr}]\,O_4$ (Amersham-Buchler, Braunschweig, Germany) and washed 4 times and then resuspended in medium in a concentration of 2 \times $10^5/\mathrm{ml}$. The effector cells were adjusted to a concentration of 5 x $10^6/\text{ml}$. Increasing amounts of CTLs in 100 μl were titrated to 10^4 target cells/well or cavity in 50 µl. 50 µl antibodies were added to each well. The entire test was prepared three times and incubated at 37°C for 4 h. 100 µl of the supernatant were collected and tested for [51Cr] release in a gamma counter (Cobra Auto Gamma; Canberra Packard, Dreieich, Germany). The maximum release was determined by incubation of the target cells in 10 % SDS, and the spontaneous release was determined by incubation of the cells in medium alone. The specific lysis (%) was calculated as: (experimental release - spontaneous release) / (maximum release - spontaneous release) x 100.

Example 6: Construction of the plasmids pDISC5-LL and pDISC5-SL for the expression of bivalent, bispecific and/or tetravalent, bispecific F_{ν} antibody constructs in bacteria by high cell density fermentation

Expression vectors were prepared which contained the hok/sok plasmid-free cell suicide system and a gene which codes for the Skp/OmpH periplasmic factor for a greater production of recombinant antibodies. The skp gene was amplified by PCR using the primers skp-1, 5'-CGA ATT CTT AAG ATA AGA AGG AGG

TTA TTG TGA AAA AGT GGT TAT TAG CTG CAG G and skp-2, 5'-CGA ATT AAG CTT CAT TAT TTA ACC TGT TTC AGT ACG TCG G using the plasmid pGAH317 (Holck and Kleppe, 1988, Gene 67, 117-124). The resulting PCR fragment was cleaved by AflII and HindIII and inserted in the AflII/HindIII-linearized plasmid pHKK (Horn et al., 1996, Appl. Microbiol. Biotechnol. 46, 524-532) so as to obtain the vector pSKK. The genes obtained in the plasmids pDISC3x19-LL and pDISC3x19-SL and coding for the scFv antibody constructs were amplified by means of the primers fe-1, 5'-CGA ATT TCT AGA TAA GAA GGA GAA ATT AAC CAT GAA ATA CC and fe-2, 5'-CGA ATT CTT AAG CTA TTA GTG ATG GTG ATG GTG ATG TGA G. The XbaI/AflII-cleaved PCR fragments were inserted in pSKK before the skp insert so as to obtain the expression plasmids pDISC5-LL and pDISC6-SL, respectively, which contain tri-cistronic operons under the control of the lac promoter/operator system (cf. figs. 9, 10).

SEQUENCE RECORD

- (1) GENERAL INDICATIONS:
 - (i) APPLICANT:
 - (A) NAME: Deutsches Krebsforschungszentrum
 - (B) STREET: Im Neuenheimer Feld 280
 - (C) TOWN: Heidelberg
 - (E) COUNTRY: Germany
 - (F) POSTAL CODE: 69120
 - (ii) TITLE OF THE INVENTION: Multivalent Antibody Constructs
 - (iii) NUMBER OF SEQUENCES: 17
 - (iv) COMPUTER-READABLE VERSION:
 - (A) DATA CARRIER: floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, version #1.30 (EPA)
- (2) INDICATIONS AS TO SEQ ID NO: 1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1698 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: genome DNA
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 28..1689
 - (ix) FEATURE:
 - (A) NAME/KEY: mat peptide
 - (B) POSITION: 28..1689
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCC Ala	GCT Ala 10	GGC Gly	TTG Leu	CTG Leu	CTG Leu	CTG Leu 15	GCA Ala	GCT Ala	CAG Gln	CCG Pro	GCC Ala 20	ATG Met	GCG Ala	CAG Gln	GTG Val	99
CAA Gln 25	CTG Leu	CAG Gln	CAG Gln	TCT	GGG Gly 30	GCT Ala	GAA Glu	CTG Leu	GCA Ala	AGA Arg 35	CCT Pro	GGG Gly	GCC Ala	TCA Ser	GTG Val 40	147
AAG Lys	ATG Met	TCC Ser	TGC Cys	AAG Lys 45	GCT Ala	TCT Ser	GGC Gly	TAC Tyr	ACC Thr 50	TTT Phe	ACT Thr	AGG Arg	TAC	ACG Thr 55	ATG Met-	195
CAC His	TGG Trp	GTA Val	AAA Lys 60	CAG Gln	AGG Arg	CCT Pro	GGA Gly	CAG Gln 65	GGT Gly	CTG Leu	GAA Glu	TGG Trp	ATT Ile 70	GGA Gly	TAC Tyr	243
ATT Ile	AAT Asn	CCT Pro 75	AGC Ser	CGT Arg	GGT Gly	TAT Tyr	ACT Thr 80	AAT Asn	TAC Tyr	AAT Asn	CAG Gln	AAG Lys 85	TTC Phe	AAG Lys	GAC Asp	291
AAG Lys	GCC Ala 90	ACA Thr	TTG Leu	ACT Thr	ACA Thr	GAC Asp 95	AAA Lys	TCC Ser	TCC Ser	AGC Ser	ACA Thr 100	GCC Ala	TAC Tyr	ATG Met	CAA Gln	339
CTG Leu 105	AGC Ser	AGC Ser	CTG Leu	ACA Thr	TCT Ser 110	GAG Glu	GAC Asp	TCT Ser	GCA Ala	GTC Val 115	TAT Tyr	TAC Tyr	TGT Cys	GCA Ala	AGA Arg 120	387
TAT Tyr	TAT Tyr	GAT Asp	GAT Asp	CAT His 125	TAC Tyr	AGC Ser	CTT Leu	GAC Asp	TAC Tyr 130	TGG Trp	GGC Gly	CAA Gln	GGC Gly	ACC Thr 135	ACT Thr	435
CTC Leu	ACA Thr	GTC Val	TCC Ser 140	TCA Ser	GCC Ala	AAA Lys	ACA Thr	ACA Thr 145	CCC Pro	AAG Lys	CTT Leu	GGC Gly	GGT Gly 150	GAT Asp	ATC Ile	483
TTG Leu	CTC Leu	ACC Thr 155	CAA Gln	ACT Thr	CCA Pro	GCT Ala	TCT Ser 160	TTG Leu	GCT Ala	GTG Val	TCT Ser	CTA Leu 165	GGG Gly	CAG Gln	AGG Arg	531
GCC Ala	ACC Thr 170	ATC Ile	TCC Ser	TGC Cys	AAG Lys	GCC Ala 175	AGC Ser	CAA Gln	AGT Ser	GTT Val	GAT Asp 180	TAT Tyr	GAT Asp	GGT Gly	GAT Asp	579
AGT Ser 185	TAT Tyr	TTG Leu	AAC Asn	TGG Trp	TAC Tyr 190	CAA Gln	CAG Gln	ATT Ile	CCA Pro	GGA Gly 195	CAG Gln	CCA Pro	CCC Pro	AAA Lys	CTC Leu 200	627
CTC Leu	ATC Ile	TAT Tyr	GAT Asp	GCA Ala 205	TCC Ser	AAT Asn	CTA Leu	GTT Val	TCT Ser 210	GGG Gly	ATC Ile	CCA Pro	CCC Pro	AGG Arg 215	TTT Phe	675
AGT Ser		Ser					Asp						CAT His 230			723

			Asp										ACT		GAT Asp	771
		Thr											CGG Arg		GAT Asp	819
	Ala												AGC Ser		GGT Gly- 280	867
													CAG Gln			915
GCT Ala	GAG Glu	CTG Leu	GTG Val 300	AGG Arg	CCT Pro	GGG Gly	TCC Ser	TCA Ser 305	GTG Val	AAG Lys	ATT Ile	TCC Ser	TGC Cys 310	AAG Lys	GCT Ala	963
													AAG Lys			1011
													GGA Gly			1059
													CTG Leu			1107
													CTA Leu			1155
													ACG Thr 390			1203
													ACC Thr			1251
													GAT Asp			1299
													GAG Glu			1347
													AAC Asn			1395

					ACC Thr						1443
					GTC Val						1491
					ACA Thr					GCC Ala.	1539
					CAG Gln 510						1587
					ATA Ile						1635
					TCA Ser						1683
CAT His	CAC His	TAAT	CTAC	GΑ							1698

(2) INDICATIONS AS TO ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 554 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys 85 90 95 Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp 100 105 110 Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu 115 120 125 Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Lys Leu Gly Gly Asp Ile Leu Leu Thr Gln Thr Pro Ala Ser 145 150 155 160 Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser 165 170 175 Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln 180 $$185\ \ \, 190\ \ \, 190$ Ile Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu 195 200 205 Val Ser Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp 210 215 220 Phe Thr Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr 225 230 235 240 His Cys Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr 245 250 255 Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Ala Ala Gly Gly Gly Gly 260 265 270 Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser 275 280 285 Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser 290 295 300 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr 305 310 315 320 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile 325 330 335 Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe 340 345 350 Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr 355 360 365
 Met
 Gln
 Leu
 Ala
 Ser
 Clu
 Asp
 Ser
 Ala
 Val
 Gly
 Arg
 Tyr
 Phe
 Cys
 Ala
 Met
 Asp
 Asp
 Asp
 Glu
 Thr
 Thr
 Thr
 Val
 Gly
 Arg
 Tyr
 Tyr
 Ala
 Met
 Asp
 Asp</th

- (2) INDICATIONS AS TO ID NO: 3:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1653 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: genome DNA
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 28..1644

	(ix)	(A)	NAME POSI	TION	1: 28	31									
	(xi)	SE	QUE	ICE I	DESC	RIPT	ION	: SE	QID	NO:	: 3:					
GAA	TTCA	TTA	AAGA	.GGAG	AA A	TTAA							CT A			
GCC Ala	GCT Ala 10	Gly	TTG	CTG Leu	CTG	CTG Leu 15	Ala	GCT Ala	CAG Gln	CCG	GCC Ala 20	Met	GCG	CAG Gln	GTG Val	. 9
CAA Gln 25	CTG Leu	CAG Gln	CAG G1n	TCT	GGG Gly 30	GCT	GAA Glu	CTG Leu	GCA Ala	AGA Arg 35	Pro	GGG Gly	GCC Ala	TCA Ser	GTG Val 40	14
AAG Lys	ATG Met	TCC Ser	TGC Cys	AAG Lys 45	GCT Ala	TCT Ser	GGC Gly	TAC Tyr	ACC Thr 50	TTT	ACT Thr	AGG Arg	TAC Tyr	ACG Thr 55	ATG Met	19
CAC His	TGG Trp	GTA Val	AAA Lys 60	Gln	AGG Arg	Pro	GGA Gly	CAG Gln 65	GGT Gly	CTG Leu	GAA Glu	TGG Trp	ATT Ile 70	GGA Gly	TAC Tyr	24
ATT Ile	AAT Asn	CCT Pro 75	AGC Ser	CGT Arg	GGT Gly	TAT Tyr	ACT Thr 80	AAT Asn	TAC Tyr	AAT Asn	CAG Gln	AAG Lys 85	TTC Phe	AAG Lys	GAC Asp	29
AAG Lys	GCC Ala 90	ACA Thr	TTG Leu	ACT Thr	ACA Thr	GAC Asp 95	AAA Lys	TCC Ser	TCC Ser	AGC Ser	ACA Thr 100	GCC Ala	TAC Tyr	ATG Met	CAA Gln	33
													TGT Cys			38
													GGC Gly			43
CTC Leu	ACA Thr	GTC Val	TCC Ser 140	TCA Ser	GCC Ala	AAA Lys	ACA Thr	ACA Thr 145	CCC Pro	AAG Lys	CTT Leu	GGC Gly	GGT Gly 150	GAT Asp	ATC Ile	48
TTG Leu	CTC Leu	ACC Thr 155	CAA G1n	ACT Thr	CCA Pro	GCT Ala	TCT Ser 160	TTG Leu	GCT Ala	GTG Val	TCT Ser	CTA Leu 165	GGG Gly	CAG Gln	AGG Arg	53:
Ala	ACC Thr 170	ATC Ile	TCC Ser	TGC Cys	AAG Lys	GCC Ala 175	AGC Ser	CAA Gln	AGT Ser	GTT Val	GAT Asp 180	TAT Tyr	GAT Asp	GGT Gly	GAT Asp	57

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AGT	TAT	TTG	AAC	TGG	TAC	CAA	CAG	ATT	422	CCA	CAG	CCA	ccc	454	CTC	627
Ser 185	Tyr	Leu	Asn	Trp	Tyr 190	Gln	Gln	Ile	Pro	Gly 195	Gln	Pro	Pro	Lys	Leu 200	027
CTC Leu	ATC Ile	TAT Tyr	GAT Asp	GCA Ala 205	TCC Ser	AAT Asn	CTA Leu	GTT Val	TCT Ser 210	GGG Gly	ATC Ile	CCA Pro	CCC Pro	AGG Arg 215	TTT Phe	675
AGT Ser	GGC Gly	AGT Ser	GGG Gly 220	TCT Ser	GGG Gly	ACA Thr	GAC Asp	TTC Phe 225	ACC Thr	CTC Leu	AAC Asn	ATC Ile	CAT His 230	CCT Pro	GTG Val	723
GAG Glu	AAG Lys	GTG Val 235	GAT Asp	GCT Ala	GCA Ala	ACC Thr	TAT Tyr 240	CAC His	TGT Cys	CAG Gln	CAA Gln	AGT Ser 245	ACT Thr	GAG Glu	GAT Asp	771
CCG Pro	TGG Trp 250	ACG Thr	TTC Phe	GGT Gly	GGA Gly	GGC Gly 255	ACC Thr	AAG Lys	CTG Leu	GAA Glu	ATC Ile 260	AAA Lys	CGG Arg	GCT Ala	GAT Asp	819
GCT Ala 265	GCG Ala	GCC Ala	GCT Ala	GGT Gly	GGC Gly 270	CCA Pro	GGG Gly	TCG Ser	CAG Gln	GTG Val 275	CAG Gln	CTG Leu	CAG Gln	CAG Gln	TCT Ser 280	867
GGG Gly	GCT Ala	GAG Glu	CTG Leu	GTG Val 285	AGG Arg	CCT Pro	GGG Gly	TCC Ser	TCA Ser 290	GTG Val	AAG Lys	ATT Ile	TCC Ser	TGC Cys 295	AAG Lys	915
												TGG Trp				963
												TGG Trp 325				1011
												GCC Ala				1059
												AGC Ser				1107
TCT Ser	GAG Glu	GAC Asp	TCT Ser	GCG Ala 365	GTC Val	TAT Tyr	TTC Phe	TGT Cys	GCA Ala 370	AGA Arg	CGG Arg	GAG Glu	ACT Thr	ACG Thr 375	ACG Thr	1155
												CAA Gln				1203
	Thr					Lys						GGC Gly 405				1251

Q.

CTC Leu 410									1299
ACC Thr									1347
CAG Gln									1395
AAA Lys									1443
ACC Thr									1491
ACT Thr 490									1539
GGG Gly									1587
GAA Glu									1635
CAT His	TAA	CTAC	GA.						1653

(2) INDICATIONS AS TO ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 539 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala 1 $$ 15 $$ 10 $$ 15

Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala Glu 20 25 30

Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly $_{35}^{}$

Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly 50Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu 115 120 125 Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr 130 140 Thr Pro Lys Leu Gly Gly Asp Ile Leu Leu Thr Gln Thr Pro Ala Ser 145 150 155 160 Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser 165 170 175 Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln 180 185 190 Ile Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu 195 . 200 205 Val Ser Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp 210 215 220 Phe Thr Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr 225 230 235 240 His Cys Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr 245 250 Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Ala Ala Gly Gly Pro Gly $260 \\ 260 \\ 265$ Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly 275 280 285 Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser 290 295 300 Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp 305 \$310\$Ile Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys

(2) INDICATIONS AS TO ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
- (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"
- (iii) HYPOTHETICAL: no
 (iv) ANTISENSE: no
- (xi) SEQUENCE DESCRIPTION: SEO ID NO: 5:

TATATACTGC	AGCTGCACCT GCGACCCTGG GCCACCAGCG GCCGCAGCAT CAGCCCG	57
	DICATIONS AS TO ID NO: 6:	3 '
(ii: (iv) (xi)	KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer" 1) HYPOTHETICAL: no ANTISENSE: no SEQUENCE DESCRIPTION: SEQ ID NO: 6: CAGGTGCAAC TGCAGCAGTC TGGGGCTGAA CTGGC	45
(i) (ii) (iii (iv)	CATIONS AS TO ID NO: 7: SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer") HYPOTHETICAL: no ANTISENSE: no SEQUENCE DESCRIPTION: SEQ ID NO: 7:	

GGTCGACGTT AACCGACAAA CAACAGATAA AACG

34

(2)	1	NDIC	CATIO	ONS	AS I	'O II	ON C	: 8:								
	(i)	SEQ	UENC	E CI	IARA	CTER	IST	ics:							
			(A)	L	ENGT	H: 3	48 h	ase	pai	rs						
			(B)	K	IND:	nuc	leot	ide								
			(C)	S:	ran	D TY	PE:	sin	gle	stra	and					
			(D)	T	OPOL	OGY:	lir	near								
	(ii)	KIN	D OF	MO1	LECU	LE:	geno	ome	DNA						
	(iii)	HY:	POTH	ETIC	AL:	no									
	(iv)	ANT	ISEN	SE:	no									-	
	(ix)	FEA	TURE	::											
			(A)	N	AME/	KEY:	CDS	3								
			(B)	P	SIT	ION:	1	348								
	(ix)	FEA	TURE	:											
			(A)	N/	ME/	KEY:	mat	_pe	ptid	e						
			(B)		SIT											
	(xi)	SEQ	UENC	E DE	SCR	IPTI	ON:	SEQ	ID	NO:	8:				
ATG	AGA	TTT	CCT	TCA	ATT	TTT	ACT	GCT	GTT	тта	TTC	GCA	GCA	TCC	TCC	4.8
														Ser		•••
1				5					10					15		
ccs	mm v	com	com	001	cmc	330	3.00			~	~~			GCA		
Ala	Leu	Ala	Ala	Pro	Val	Asn	Thr	Thr	Thr	GAA	ASD	GAA	Thr	Ala	Gln	96
			20			*****		25	****				30		0111	
														GAT		144
TTE	Pro	35	GIU	Ala	vai	TIE	40	ıyr	Ser	Asp	Leu	G1u 45	GIY	Asp	Phe	
							40					43				
GAT	GTT	GCT	GTT	TTG	CCA	TTT	TCC	AAC	AGC	ACA	AAT	AAC	GGG	TTA	TTG	192
Asp	Val	Ala	Val	Leu	Pro		Ser	Asn	Ser	Thr		Asn	Gly	Leu	Leu	
	50					55					60					
ششت	ATA	AAT	ACT	аст	ATT	GCC	AGC	ልጥጥ	COT	ССТ	222	CAA	CAL	GGG	CT A	240
Phe	Ile	Asn	Thr	Thr	Ile	Ala	Ser	Ile	Ala	Ala	Lys	Glu	Glu	Gly	Val	2.00
65					70					75				-	80	
mom	omo	C2.C			~~~				~~~					CTG		000
Ser	LAN	GAG	Tare	Ara	GAG	Ala	GAA	Ala	Clu	Pho	CAG	1707	CAA	Leu	CAG	288
562	neu	Olu	LJ 5	85	oru	AIG	GIU	AIG	90	rne	GIII	VOI	GIII	95	GIII	
														ATG		336
Gln	Ser	Gly		Glu	Leu	Ala	Arg		Gly	Ala	Ser	Val		Met	Ser	
			100					105					110			
TGC	AAG	GCT	TCT													348
		Ala														
		115														

2) INDICATIONS AS TO ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 amino acids

(B) KIND: amino acid (D) TOPOLOGY: linear

(ii) KIND OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser 1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
20 25 30

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu 50 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val 65 70 80 Ser Leu Glu Lys Arg Glu Ala Glu Ala Glu Phe Gln Val Gln Leu Gln 85 90 95

Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser 100 105 110

Cys Lys Ala Ser

- (2) INDICATIONS AS TO ID NO: 10:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 354 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: genome DNA
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 1..354
 - (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) POSITION: 1..354
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATG Met	AGA Arg	TTT Phe	CCT	TCA Ser 5	ATT Ile	TTT Phe	ACT Thr	GCT Ala	GTT Val 10	TTA Leu	TTC Phe	GCA Ala	GCA Ala	TCC Ser 15	TCC Ser	4.8
				CCA Pro												96
				GCT Ala											TTC T	144
				TTG Leu												192
				ACT Thr												240
				AGA Arg 85												288
				GGG Gly												336
				GCT Ala												354
2)	IND	S) ()	EQUE A) 3)	S AS NCE LENG KING	CHAF GTH: D: ai	RACTI 118 mino	ERIS ami	TICS		s						

- (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser 1 15

The Pro Ala Glu Ala Val The Gly Tyr Ser Asp Leu Glu Gly Asp Phe $_{35}^{\rm He}$

42

Asp	Val 50	Ala	Val	Leu	Pro	Phe 55	Ser	Asn	Ser	Thr	Asn 60	Asn	Gly	Leu	Leu
Phe 65	Ile	Asn	Thr	Thr	Ile 70	Ala	Ser	Ile	Ala	Ala 75	Lys	Glu	Glu	Gly	Val 80
Ser	Leu	Glu	Lys		Glu			Ala			Met		Gln	Val 95	Gln
Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lvs

105

100 Met Ser Cys Lys Ala Ser 115

- (2) INDICATIONS AS TO ID NO: 12:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"
 - (iii) HYPOTHETICAL: no (iv) ANTISENSE: no
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TCACACAGAA TTCTTAGATC TATTAAAGAG GAGAAATTAA CC

(2) INDICATIONS AS TO ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: other nucleic acid
- (A) DESCRIPTION: /desc = "primer"
- (iii) HYPOTHETICAL: no
- (iv) ANTISENSE: no
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

T C TOTAL CONTRACTOR CONTRACT

AGC.	ACACGAT ATCACCGCCA AGCTTGGGTG TTGTTTTGGC	40
(2)	INDICATIONS AS TO ID NO: 14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 43 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIFTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14	
AGC	ACACAAG CTTGGCGGTG ATATCTTGCT CACCCAAACT CCA	43
	INDICATIONS AS TO ID NO: 15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 57 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer"	
	(iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15	
AGCA	ACACTET AGAGACACA AGATETTTAG TGATGGTGAT GGTGATGTGA GTTTAGG	57
(2)	INDICATIONS AS TO ID NO: 16: (i) SEQUENCE CHARACTERISTICS: (A) LENCTH: 33 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single_strand (D) TOPOLOGY: linear	

(ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID No: 16:	
4	
CAGCCGGCCA TGGCGCAGGT GCAACTGCAG CAG	33
(2) INDICATIONS AS TO ID NO: 17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 102 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:	
TATATACTGC AGCTGCACCT GGCTACCACC ACCACCGGAG CCGCCACCAC CGCTACCACC	60
GCCGCCAGAA CCACCACCAC CAGCGGCCGC AGCATCAGCC CG	102

Official File: PCT/DE99/01350 Attorney's File: K 2675

Amended Claims

- 1. A multivalent F_{ν} antibody construct having at least four variable domains which are linked with one another via the peptide linkers 1, 2 and 3, wherein the peptide linkers 1 and 3 have 0 to 10 amino acids.
- 2. The F_{ν} antibody construct according to claim 1, wherein the peptide linkers 1 and 3 have the amino acid sequence GG.
- 3. The F_{ν} antibody construct according to claim 1 or 2, wherein the F_{ν} antibody construct is bivalent.
- 4. The F_{ν} antibody construct according to claim 3, wherein the peptide linker 2 has 11 to 20 amino acids.
- 5. The F_{ν} antibody construct according to claim 3 or 4, wherein the peptide linker 2 has the amino acid sequence $(G_4S)_4$.
- 6. The F_{ν} antibody construct according to claim 1 or 2, wherein the F_{ν} antibody construct is tetravalent.
- 7. The F_{ν} antibody construct according to claim 6, wherein the peptide linker 2 has 3 to 10 amino acids.

- 8. The E_{ν} antibody construct according to claim 6 or 7, wherein the peptide linker 2 comprises the amino acid sequence GGPGS.
- 9. The F_{ν} antibody construct according to any of claims 1 to 8, wherein the F_{ν} antibody construct is multispecific.
- 10. F_{ν} antibody construct according to claim 9, wherein the F_{ν} antibody construct is bispecific.
- 11. The F_{ν} antibody construct according to any of claims 1 to 8, wherein the F_{ν} antibody construct is monospecific.
- 12. A method of producing the multivalent F_{ν} antibody construct according to any of claims 1 to 11, wherein DNAs coding for the peptide linkers 1, 2 and 3 are ligated with DNAS coding for the four variable domains of an F_{ν} antibody construct such that the peptide linkers link the variable domains with one another and the resulting DNA molecule is expressed in an expression plasmid.
- 13. Expression plasmid coding for the multivalent F_{ν} antibody construct according to any of claims 1 to 11.
- 14. The expression plasmid according to claim 13, namely pDISC3x19-LL.
- 15. The expression plasmid according to claim 13, namely pDISC3x19-SL.
- 16. The expression plasmid according to claim 13, namely $\ensuremath{\text{pPIC-DISC-LL}}.$

- 17. The expression plasmid according to claim 13, namely $\ensuremath{\text{pPIC-DISC-SL}}.$
- 18. The expression plasmid according to claim 13, namely ${\tt pDISC5-LL.}$
- 19. The expression plasmid according to claim 13, namely pDISC6-SL. $\mbox{ _}$
- 20. Use of the multivalent F_{ν} antibody construct according to any of claims 1 to 11 for the diagnosis and/or treatment of diseases.
- 21. Use according to claim 20, wherein the diseases are viral, bacterial or tumoral diseases.

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EcoRI RBS PelBiesoer Nool
1 GAATTEATTAAAGAGGAAAATTAACEATGAAAATACOTATTAGUTTAGGGAAGEGGGTTGGGTTGGGTGGGTGGGTGAGTTGAGTTGAGTTGGGAATTG
19 M K 7 T L P T A A A S L L L L A A Q P A M Frame-HI VH anti-CO3
92 CGCAGGTGCAACTGCAGCAGTCTGGGGCTGAACTGGGCAACACCTGGGGCCTCAGTGAAGATGTTGTTGGTAAGATCTTTTTTTT
22° A C V Q L Q O S G A E L A R P G A S 7 K M S C K A S G Y T F T COR-H1 Frame-H2 COR-H2
183 TAGGTACACGATGCACTGGGTAAACAGAGGCCTGGACAGGGTTTGGAATGCATTGGATGCATTGATACCTAGCCGTGGTTATAC
25, 3 A L W H M A K O K S 2 O O T E M I O A I W S 2 U A A A
Frame-H3 257 TAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGCACAACTCTTCCAGCCACAGCCTACATGAGTGAG
30° N Y N Q X F X D X A T L T T D X S S S T A Y N Q L S S L T
COB-H3 Frame-int
154 ATCTOROGRACTETOCROTETRATTACAGGATATACAGGCCTTGACTACTACGGCCCLAGGCCCCAGGCCCCCCCCCC
CH1 Linker 1 Frame-1 1 VI aggi-Chia
440 CASTCTCCTCAGCCAAACAACAACACCTCGGGGGGTCATACCTCACCCAAACCCCACCCCCCCC
138 T V S S A X T T P X L G G D T L L T Q T P A S L A 7 S L G Q COR-L: Frame-L2
530 GGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATTTGAACTGCTACCACCACACTATTCAGGAC
168) RATISCKASQSVDYDGDSYLMWYQQIPG CDR-12 Frame-13
CDR-L2 Frame-L3 614 AGCCACCCA-ACTCCTCATCTTATGATGCATCCAATCTTAGTTTCTGGGATCCCACCCCACGTTTAGTGCACCAGTGGGTCTGGGACAGTCTT
1967Q P P K L L I Y D A S N L V S G I P P R F S G S G S G T D F
COR-L3 Frame-L4 702 CACCOTCAACATCCATCCAGGAGAGGTGGATGCTGCAACCTATCAGGAAGGA
205) T L N I H P 7 E K Y D A A T Y H C Q Q S T E D P N T F C G
Ckapps Not! Linker 2
790 GCCACCAACTGGAATCAAACTGGATTGTCCCCCCCTGGTGGTGGTGGTTGGT
Pvull Frame-H1 VH anti-CD19
874 TCCGGTGGTGGTGGTAGCCAGCTGCAGCAGCTCCAGCAGCTGAGCTGAGCTGAGCTGAGCTGCAGCTGAAGATTTCCTGCAAGG 283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X
CORH1 Frame-H2 CORH2
962 CTTCTCGCTLATCACTTCACTAGCTACTGGATGAACTGGGTGAAGCAGACCCTGGACAGGGTCTTCAGTGGATTGGACAGATTTGGC
312 A 5 G Y A F S 5 Y W M N W V K Q R P 3 Q G L E W I G Q I W
313PA S G Y A F S S Y W M N W V X Q R P G Q G L E W E G Q E W P M C R P G Q E W P M C R P G Q E W P M C R P M P M P M P M P M P M P M P M P M P
3.22% S G Y A F S S Y M M M M W Y R R P D Q G L E M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T G G T M T M
3.12% S G Y A F S S Y W M N W V X G R P D G G L E W D G G C Z W P S G G C E W D G G C Z W P S G G C E W D G G C Z W P S G F F S G D G D C M Y N G R F X G X A T L T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A D E S S S T A Y C D T A T A D E S S S S T A Y C D T A D E S S S S T A Y C D T A D E S S S S T A Y C D T A D E S S S S S S S S S S S S S S S S S S
312PA 5 G Y A F S 5 Y M M M M W X Q R P D G G L E W I G G I W I G R P G G L E W I G G I W I G G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G G I W I G G G I W I G G G G
3.12% 5 G Y A F S 5 Y M M N M V X Q R P D Q G L E M D G C D W 1049 <u>ETGGAGATGGTGATACTAACTAAGGAAGTTAAGGGTAAACCACCCAACCAA</u>
312PA 5 G Y A F S 5 Y M M M M W X Q R P D G G L E W I G G I W I G R P G G L E W I G G I W I G G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G I W I G G G I W I G G G I W I G G G G
3.22% S G Y A F S S Y W M N W W X Q R P D Q G L E W T G C T W T B P G T S Y W M N W W X Q R P D Q G L E W T G C T W
3129A 5 G Y A F S S Y W M N W W X Q R P D G G L E W I G G I W I G
3129A 5 G Y A F S S Y N M N N W V X Q R P D G G L E W 1 G C T W 1
3.22% S G Y A F S S Y W M N W W X Q R P D G G L E W T G G T W 1049 STEGGRATHSTEGATACTACTACTACGAGGAGTTCAAGGGTAGGCCCTCACC 1049 STEGGRATHSTEGATACTACTACTACGAGGAGTACCACCACCACCACCACCACCACCACCACCACCACCACC
3129A 5 G Y A F S S Y N M N N W V X Q R P D G G L E W I G C I W I
3.32% A 5 G Y A F S 5 Y W M N N W V X Q R P D G G L E W 1 G C 1 W 1 G C 1 W 1 G C T W
3329A 5 G Y A F S 5 Y M M N M W V K Q R P D Q G L E M I G C I M I G S I M I M I M V K Q R P D Q G L E M I G C I M I M I M I M I M I M I M I M I M I
3.23% S
3329A 5 G Y A F S 5 Y M M N M W V K Q R P D Q G L E M I G C I M I G S I M I M I M V K Q R P D Q G L E M I G C I M I M I M I M I M I M I M I M I M I
3129
3.23% S

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EcoRI RBS PalB leader	Neol
SAATTOATTAAAGAGAAGAATTAACCATGAAATTACCTATTGCCTACGGCAGCCGCTGGCTTGCTGCTGCTGCAGCACCTCACTTACT	none n
PMKYLLPTAAAGLILAAQP	2 14
* Frame-H1 VH and CD2	
92 CSCAGSTGCHACTGCAGCAGTCTGGGGCTGAACTGGGCAAGACCTGGGGCCTCAGTGAAGATGTGCTGCAAGGCTTGTGGCTACAC	7
22° A Q V Q E Q Q S S A E L A R P G A S V K M S C K A S G Y T	7 T
CDR-H1 Frame-H2 CDR-H2 →	
183 TAGGTACACGATGCACTCGGTAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGGCGGTGGT	ATAC
52) RYTHHWYXQRPGQGLEWIGYINPSFG	7 7
Frame-ri3	
267 TAATTACAATCAGAAGTTCAAGGACAAGGCAACATTGACTACAGAAAACCTTCAGGACAGCCTTACATGCAACTGAGCACG	CTGAC
	L T
CDR-H3 Frame-H4 354_ATCTGAGGACTCTGCAGTGTAGTGCAAGA <u>TGATGATGATGATGAGGCCTTGAGTAGTGGCCAAGCCCAAGCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGAAG</u>	
109° S E D S A V Y Y C A R Y Y D D H Y S L D Y W G Q G T T	10103
CH1 (inter 1 Frame.) 1 VI and CD1	
440 CAGTOTOCTCAGCCAAAACAACACTCAACCTTTGGCGGTGATATCTTGCTCACCCAAACCCCAAGCTTCTTTTGGCTGTGTCTCTAGG	20707
138 T V S S A K T T F K L G G D I L L T Q T P A S L A 7 S L S	C
COR-L1 Frames 2	
530 GGGCCACCATCTCCTGCAAGGCCAAAGTGTTGATTATGATGGTGATAGTTATTTGAACTGTACCAACAGATTC	aggac
- 1887 X T I S C X A S Q S Y D C D C D S Y L X W Y C C C ;	. 0
CDR-L2 Frame-L3	
514 AGCCACCCAAACTCCTCATGATGCATCCAATCTAGTTTCTSCCATCCCACCCAGGTTTAGTGCAGTGGGTGTGGAAC	GACTT
196°Q P P X L L 1 Y D A S N L V S J I P P R F S G S G S G I	
CDR-L3 Frame-L-	
702 CACCOTCAACATCCATCCTGTGGAGAGGTGGATGCTGCAACCTATCACTGTGAGGAAAGTACTGAGGATCGTGGAGGATCCTTGGAGGATCCTTGAGGATACGTAAGTACTGAGGATCCTTGAGGATCACTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGGATCCTTGAGAGATCACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACTTGAGATCACACACA	JIGGA
Ckappa Noti Linker 3 Pyuli Framei-Hi	3 G
790 GGCACCAAGCTGGAAATCAAACGGGTCAAGTGAAATCAAACGGGTCAGGGTCGGGCCAGGGTGCAGGTGCAGCAGCTGCAGCAGCTGCAGCAGCTGCAGCAGCTGCAGCAGCTGCAGCAGCAGCTGCAGCAGCAGCTGCAGCAGCAGCTGCAGCAGCAGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	~~~
255) G T K L E I K R A D A A A A G G P G S Q V Q L Q Q S G A	T C
VH anti-CD19 CD8-H1 Scame	-H2
879 GGTGAGGCCTGGGTCCTCAGTGAACATTTCCTGCAAGGCTTCTGGCTATGCATTCAGTAGCTACTGGATGAACTGGGTGAAGC	GAGGC
284) V R P G S S V K I S C X A S G Y A F S S Y W M N W V K C	3.
CDR-H2	
968 CTGGACAGGGTCTTGAGTGGATTGGAGATTTGGCCTGGAGATGGTGATACTAACTA	AAGCC
314°PGQGLEWIGQINPGDGDTNYNGKFKG	K A
Frame-H3	
1051 ACTOTGACTGCAGACGAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTAGCATCTCAGGACTCTCCGGGTCTACTTCTGTGC 342 T L T A D E S S S T A Y M O L S S L A S E D S A V Y F C A	
	CH1
COR-H3 Frame-H4 11/2 GGG3 GGGT3 GGGGT3 GGGCGTT3 TTT3 CT3 TGGT3 TGG3 CT3 TGGGT3 TGG3 CT3 CTC3 CTC	CHI
1142 GGGAGACTACGACGGTAGGCCGTTATTACTATGGTATGG	CAAAA
1142 GGGAGACTACGACGGTAGGCCSTTATTACTATGGTATGGACTACTGGGTCACGACCACCACCACCACCACCACCACCACCACCACCACC	CAAAA
1142 <u>GEGGGETAGACGGTAGGCGTTAFTACTAFGCTAFGCACTACTGGGCGTCAGGACTACTGGGCGTAGGACTACTGGGCGTAGGACTACGGCGTAGGACTACGGCGAGAGACAGGACTACGGACAGACA</u>	K
1142 GGASAKTIGGAGGTAGGGGTATTAGTATGGATGGATGAGTGATTAGGATAGGATTAGGATGAGTGAGTAGGATTAGGATGGAGTAGGATTAGGATGGAGTAGGATGAGGAG	CLALA K
1112 GGGGGGTAGGGGGTAGGGGGTTATTAGTAGGGTAGGGCTGGTGGGGGGGG	CAAAA K TTGCA C
1112 GGGGGGTAGGGGGTAGGGGGTTATTAGTAGGGACTAGTUSGGACTAGTUSGGACTAGTUGGGGGTAGTGGGGGGGTAGGGGGTAGGGGGGTAGGGGGGG	CAAAA K TTGCA C
1142 GAGRAGATAGGACGATAGCCGTTATTAGTAGGACTAGTGGAGTAGGACTAGTGAGAGTAGTGAGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGTAGGAGAGTAGGAGG	COALA COR-L2
1142 GRADATTAGRADGTAGGCGTTATTAGTAGGTAGGACTAGTSSGTAMAGACTAGTAGGTAGGACTAGTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGG	CAAAA K CTGCA C CDR-L2 CCAAA S X
1112 GEGRAGATAGGROGATAGGROGATAGGROGATAGGRAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	COALA CCCAA CCCAA SX
1142 GRADATTAGRADGTAGGCGTTATTAGTAGGTAGGACTAGTSSGTAMAGACTAGTAGGTAGGACTAGTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGG	COALA CCCAA CCCAA SX
1112 GRAGAGTAGGAGGTAGGAGGTAGTAGTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTAGGACTAGTTAGGACTAGTAGGACTAGTTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACTAGTAGGACT	COALA K C CDR-L2 CCAA S X CATGO D A
1142 GEGRAATTAGGAGGTTAGGAGGTTATAGTAGGACTAGTAGGACTAGTAGGAGAGAGA	COALA K C C CBR-L2 CCAA S K CATGO D A ACTICO
1112 GGGGGGGTAGGGGGTAGGGGGTTATTAGTAGGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGTTAGGACTAGGACTAGACTAGGACTAGACTAGGACTAGACTAGGACTAGACTAGGACTAGACTAGGACTAGAC	COALA K C C CBR-L2 CCAA S K CATGO D A ACTICO
1142 GEGRAATTAGGAGGTTAGGAGGTTATAGTAGGACTAGTAGGACTAGTAGGAGAGAGA	COALA K C C CBR-L2 CCAA S K CATGO D A ACTICO

A	TG.	AGA	TT	CCI	TCA	AT	TTT	TAC	TGC	TGT	TTT.	ATT	CGC.	AGC.	ATC	CTC	CG	CAT	TA	GCT	GCT	CCA	GTC	AAC	ACT	AC
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alpha-factor signal																										
A	AC.	GA	AGA!	TGA.	AAC	GGC	AC	LAAT	TCC	GGC	TGA	AGC	TG1	CAT	CGC	FT	ACT	CAC	IA.	TT	GA.	GGG	GAT	كنعلم	רבת	77
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		K	Ε	Ε	G	٧	S	L	Ε	K	R	Ε	Α			E	F			٧	Q	L				s
	VΗ	а	nti	-CI	03																					
T	GG	GC	TG	AAC	TG	GC	AAG	ACC	TG	GGG	CC	FCA	GT	SAA	GA'	rGI	cc	TG	CA	AG	3CT	TCI				
•	G	А		Ε	L	Α	P	F	•	G	Α	s	٧	K		4	S	C		K	Α	s				
	A TY	AACA AACA TTTGC V A GCTA A VH TGGG	AACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AACAGAAGA TED TTGCTGTTT VAVI GCTAAAGAAG AKE VH anti	AACAGAAGATGA TEDE TTGCTGTTTTGG VAVL CCTAAAGAAGAA AKEE VH anti-CI TGGGGCTGAAG	MARPPS AACAGAAGATGAAAC TEDET TTGCTGTTTTTGCCAT VAVLP GCTAAAGAAGAAGAGGG VH anti-CD3 TGGGGCTGAACTG	AACAGAAGATGAACGOC TEDETA TTCGGTTTTGCCATTTI VAVLPF CCTAAAGAAGAAGAAGGGGTA AKEEGV VH anti-CD3 TGGGGTGAACTGCC	M R F P S I F ANCAGANGANGANGACGCACI T E D E T A C THEOTETHIGGCATTRICCI V A V L P F S CCTAAAGAAGAAGGAGGGTATCI A K E E G V S VH anti-CD3 TGGGGCTGAATTGGCAAG	MRFPSIFT AACAGAAGATGAAACGGCACAAAA TEDETA QI TTGCTGTTTTGCCATTTTCCAACA VAVLPFSN CCTAAAGAAGAAGAGGGGGTATCTCTC AKEEGVSL VH anti-CD3 TGGGGCTGAACTGCCAAGACC	AACAGAAGATGAAACGGCACAAATTCC T E D E T A Q I F TTGCTGTTTTGCCATTTTCCAACAGCA V A V L P F S N S CCTAAAGAAGAGAGGGGTATCTCTCGAG A K E E G V S L E VH anti-CD3 TGGGGCTGAACTGCAAGACCTG	M R F P S I F T A V AACAGAAGATTAAACGGCACAAATTCCGGG T E D E T A Q I P A TTGCTGTTTTGCCACTTTTCCAACAGCACA V A V L P F S N S T GCTAAAGAAGAAGAGGGGGTATCTCTCGAGAAA A K E E G V S L E K VH anti-CD3 TGGGGCTGAACTGGCAAGACCTGGGG	M R F P S I F T A V L all AACAGAAGATGAAACGGCACAAATTCGGGTTA T E D E T A Q I P A E TTGCTGTTTTGGCATTTTCAACAGGACAAATA V A V L P F S N S T N CCTAAAGAAGAAGAAGGACAAATA A K E E G V S L E K R VH anti-CD3 TGGGGCTGAACTGGACACAGGCCT	M R F P S I F T A V L F siphs and analysis of the property of	M R F P S I F T A V L F A alpha-fac AACAGAAGATGAAACGGCACAAATTCCGGCTTAAGCTO! T E D E T A Q I P A E A V TTGCTGTTTTTGGCATTTTCCAACAGACAAATAACGGT V A V L P F S N S T N N G CCTAAAGAAGAAGAGGGGTTATCTCTCAGGAAAAGAAGGGGT A K E E G V S L E K R E A VH anti-CD3 TGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTT	M R F P S I F T A V L F A A sipha-factor ACCAGAAGATGAACGGCACAAATTCCGGCTTGAACTGTCAA T E D E T A Q I P A E A V I TTCCTGTTTTGCAACTTTCCAACAGCAAAAAAACAGGTTGA V A V L P F S N S T N N G L GCTAAAGAAGAAGAGGGGGTTAACGGGTTGAA A K E E G V S L E K R E A E VH anti-CD3 TGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAA	M R F P S I F T A V L F A A S alpha-factor signa-factor	M R F P S I F T A V L F A A S S alpha-factor signa AACAGAAGATGAAACGGCACAAATTCCGCCTGAAGCTGAAGCTGTATCGTT T E D E T A Q I P A E A V I G TTCCTGTTTTGCCATTTTCCAACAGCACAATTAAGGGGTTATTGTTT V A V L P F S N S T N N G L F CCTAAAGAAGAAGAGGGGTTATTCTCTCAAGAAAAGAA	M R F P S I F T A V L F A A S S / alpha-factor signal Anchonagatgahacocchahattcocchatacturcattcattacturcattcattacturcattcattacturcattcattacturcattcattacturcattcattacturcattcattacturcattcattacturcattacturcattacturcattcattcattcattcattcattcattcattcattcat	M R F P S I F T A V L F A A S S A alpha-factor signal and analysis of the property of the prop	M R F P S I F T A V L F A A S S A L alpha-factor signal AACAGAAGATGAAACGGCACAAATTCGGCGTAAACTCTCATCAGAA T E D E T A Q I P A E A V I G Y S D TTCGTGTTTTGCCATTTTCCAACAGCACAAATTAGGGGTTATTGTTTATAAATAC V A V L P F S N S T N N G L F I N T EcoRI CCTAAAGAAGAAGAAGGAGTATCTCCTCAAAAAAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGA	M R F P S I F T A V L F A A S S A L A alpha-factor signal AACAGAAGATGAAACGGCACAAATTCGGGCTGAAGCTTACTCACGATTM T E D E T A Q I P A E A V I G Y S D L TTGCTGTTTTGGCAATTTTCCAACAGGACAAATAACGGGTTATTGTTTATAAATACTM V A V L P F S N S T N N G L L F N N T T ECORI CCTAAAGAAGAAGAGGGGTTCTCTCCAGAAAAGAGAGGGTGAAGTGAATTCCAGGTG A K E E G V S L E K R E A E A E F Q V VH anti-CD3 TGGGGCTGAACTGGAGACCTGAAGATGAAGATGCTGAAGT TGGGGCTGAACTGAA	M R F P S I F T A V L F A A S S A L A A A A A A A A A A A A A A	M R F P S I F T A V L F A A S S A L A A P alpha-factor signal AACAGAAGATGAACGGCACAAATTCGGCTAAAGACTTGCATTACTTAC	M R F P S I F T A V L F A A S S A L A A P V A I P A A S S A L A A P V A I P A A S S A L A A P V A I P A A S S A L A A P V A I P A A S S A L A A P V A I P A	M R F P S I F T A V L F A A S S A L A A P V N alpha-factor signal AACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTTATCGATGCTAACGGCATTTATCAACGGCATTTATCAACAGCACATTATCGATTTAGAACGGCATTTATCAACAGCACAAATTAACGGCTTATTGTTTATAAATACTACTACTATCAACACAAATTAACGACTTATTGTTTATAAATACTACTACTATCAACACAAATTAACGACTTATTGTTATAAATACTACTACTATCAACACAAATTAACAACAAAAAA	M R F P S I F T A V L F A A S S A L A A P V N T alpha-factor signal ancagangangancachantecosterangettantachangantanagangantecosterangantantachangangangantecosterangantachangangangantecosterangantachangangantachantecoscantecosterangantachant

FIGURE 7

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	alpha-factor signal																										
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1235	CA	GT	TO	GGG	GC	TGA	AC	rec	CA	AGA	cc	TGC	GG	CCI	CAC	TG.	AAC	JAI	GT	CC	rgc	A	AGG	CT	PCT		
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FIGURE 8

UNSCANNABLE ITEM

RECEIVED WITH THIS APPLICATION

(ITEM ON THE 10TH FLOOR ZONE 5 IN THE FILE PREPARATION SECTION)

DOCUMENT REQUIAVEC CETTE DEMANDE

NE POUVANT ÊTRE BALAYÊ

(DOCUMENT AU 10 IÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA

PRÉPARATION DES DOSSIERS)

P1-2-3-4-9-10